BD Accuri™ C6 Flow Cytometer

Mirko Corselli, PhD
BD Biosciences
Senior Scientist

The BD Accuri C6 Flow Cytometer System

- **An affordable, full-featured, easy-to-use flow cytometer**
- **Two lasers and six detectors**

BD Accuri C6 Flow Cytometer

Laser

Sample

Detectors

SSC

FSC

533 nm

585 nm

670 nm

675 nm

Statistical Analysis

D01 Tap 1
Gate: [No Gating]

D03 Groundwater
Gate: P1

Output

Ground Water Sample	Concentration (cells/mL)	Fluorescence (mean)
1	360,000	17,283
2	366,000	17,011
3	366,000	16,996
4	361,000	16,982

Flow Cytometry within Reach™

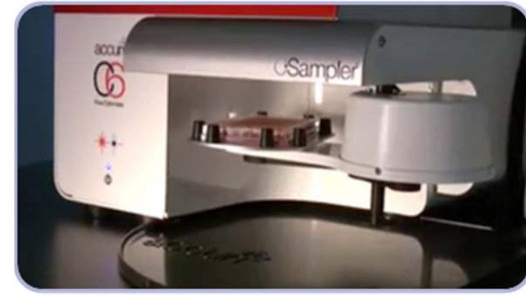
The BD Accuri™ C6 Personal Flow Cytometry Tour

Advantages of Pre-optimized Detector Settings

- **Greatly reduces the risk of lost data due to improper setup**
- **Saves time and sample**
- **No specialist training or dedicated operator required**
- **Predictable, reproducible analysis relative to the sample type and application**

Enhanced Sample Handling

- **Direct volume measurement**
- **Many types of sample tubes may be used.**
  - Flow cytometry tubes
  - Microcentrifuge tubes
  - Ninety-six-well plates with the BD CSampler™ accessory
- **Open system conducive to kinetic studies**
- **BD CSampler™ accessory for automated sample introduction**



Intuitive Software

Sample Grid

Cytometer Status

Fluidics Controls

Run Criteria

Real-Time Updates

Histogram, Dot Plot, and Density Plot Display Area

Analysis and Gating Tools

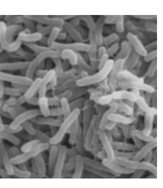
Plot Statistics

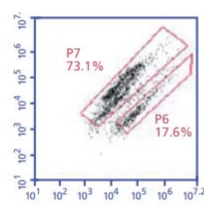
Plot 2: Sample HPB CD3-F CD4, CD45, CD8 Gated on (R1 in (P1 in all))	Count	Volume (µL)	% of This Plot	% of All	Mean CD3 FITC-A	Mean CD4 PE-A	CV CD3 FITC-A
This Plot	99,183	0.0	100.0%	43.3%	23,875.3	1,762.1	62.0%
Q3-UL	147	0.0	0.1%	0.1%	960.4	1,607.0	53.3%
Q3-UR	59,194	0.0	59.7%	25.8%	32,131.1	2,933.7	28.5%
Q3-LL	21,455	0.0	21.6%	9.4%	547.0	21.5	47.8%
Q3-LR	18,387	0.0	18.5%	8.0%	24,701.0	22.5	29.2%

Plot 3: Sample HPB CD3-F CD4, CD45, CD8 Gated on (P1 in all)	Count	Volume (µL)	% of This Plot	% of All	Mean CD3 FITC-A	Mean CD45 PE-CY7-A	CV CD3 FITC-A
This Plot	102,719	0.0	100.0%	44.8%	23,071.3	38,996.7	65.7%
R1	99,183	0.0	96.6%	43.3%	23,875.3	40,270.2	62.0%
Q1-UL	21,694	0.0	21.1%	9.5%	540.5	32,690.2	45.5%
Q1-UR	77,625	0.0	75.6%	33.9%	30,356.9	42,417.0	30.7%
Q1-LL	3,380	0.0	3.3%	1.5%	447.0	1,141.0	76.5%
Q1-LR	20	0.0	0.0%	0.0%	8,449.7	2,092.6	97.0%

A Versatile Instrument for Broad Applications

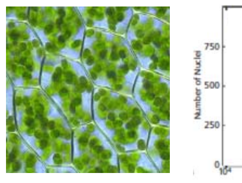
Microbiology

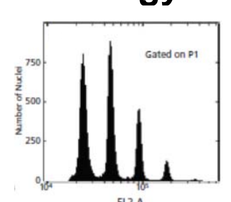




- Aquatic microbiome analysis
- Biofuel research
- Bacteria viability and concentration

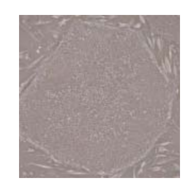
Plant Biology

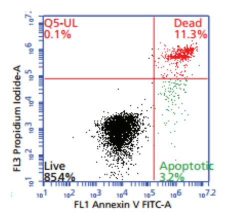




- DNA content

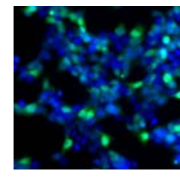
Cell Biology

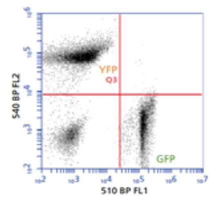




- Apoptosis
- Proliferation
- Immunophenotyping

Fluorescent Protein Analysis





- GFP,YFP
- mCherry®, RFP
- mOrange®, dTomato®

Apoptosis: Annexin V

DMSO

Camptothecin
6 µM

Live cells:
Annexin V Negative
PI Negative

Early apoptotic:
Annexin V Positive
PI Negative

Late apoptosis/dead:
Annexin V Positive
PI Positive

Jurkat cells were treated with DMSO or camptothecin for 4 hours.

Analysis of Aquatic Samples from Saginaw Bay: Fluorescence

Site 1 | Site 2 | Site 4 | Site 23

Chlorophyll *a,b*
FL3 ex: 488; em: 670 LP

Site 1: P7 50.0%, P6 42.8%
Site 2: P7 70.4%, P6 22.8%
Site 4: P7 79.1%, P6 12.2%
Site 23: P7 73.1%, P6 17.6%

Phycocyanin fluorescence
FL4 ex: 640; em: 675 ±12.5 nm

P6: Cyanobacteria
P7: Other phytoplankton

P7 Gated Plots:

Site 1 | Site 2 | Site 4 | Site 23

Phycoerythrin
FL2 ex: 488; em: 585 ±20 nm

Site 1: P8 69.4%, P9 14.2%
Site 2: P8 24.3%, P9 56.5%
Site 4: P8 65.8%, P9 18.9%
Site 23: P8 37.4%, P9 49.5%

Chlorophyll *a,b* fluorescence
FL3 ex: 488; em: 670 LP

P8: PE fluorescent
P9: Non-PE fluorescent

Four types of phytoplankton were identified by fluorescence characteristics.

Data courtesy of J.D. Bressie, PhD, NOAA, Seattle, WA

Kits and Templates on the BD Accuri C6

Category	Product Information Sheet	Brand	Kit	Cat. No.	Template
Cell Biology	BD Apoptosis Kits and Templates	BD Pharmingen™	Annexin V FITC Apoptosis Detection Kit II	556570	Download
		BD Pharmingen™	Annexin V PE Apoptosis Detection Kit I	559763	Download
		BD™	MitoScreen (JC-1) Kit	551302	Download
		BD Pharmingen™	Caspase-3 PE Assay Kit	550914	Download
		BD Pharmingen™	Caspase-3 FITC Assay Kit	550480	Download
	BD Cell Cycle and DNA Kits and Templates	BD Cycletest™ Plus	DNA Reagent Kit	340242	Download
		BD Pharmingen™	FITC BrdU Flow Kit	559619	Download
		BD Pharmingen™	APC BrdU Flow Kit	552598	Download

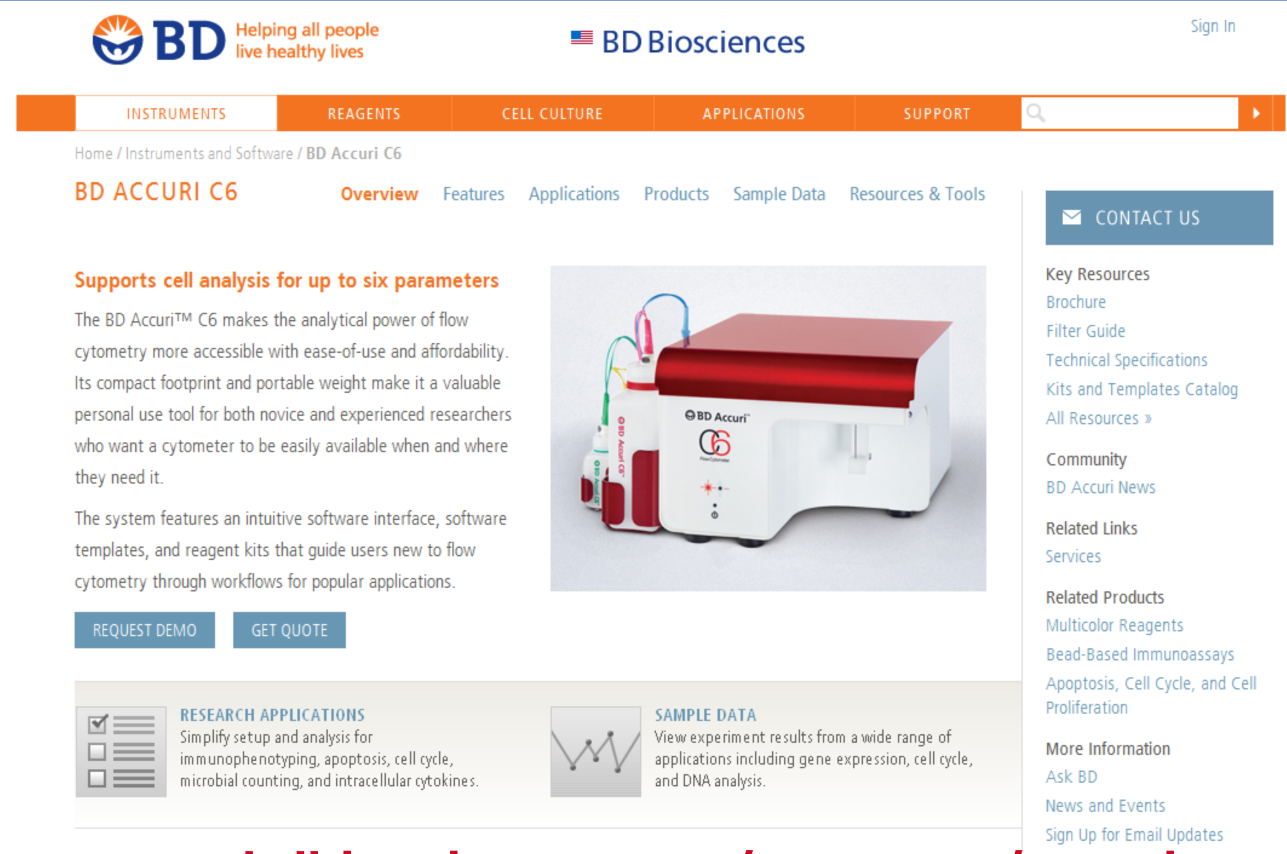
BD Accuri C6 Promotion and Personal Flow Cytometry Tour

Note:
US Region Only
Promotion Period: Oct 1, 2014 – Dec 31, 2014

The BD Accuri C6 Personal Flow Cytometry Tour

- **Introduction to Flow Cytometry**
- **Cancer and Cell Biology Applications**
- **Microbial Analysis**

For Additional Information…



www.bdbiosciences.com/resources/accuri

Technical Support:

Ph: 877-232-8995, Prompt 3, 2

email: ResearchApplications@bd.com

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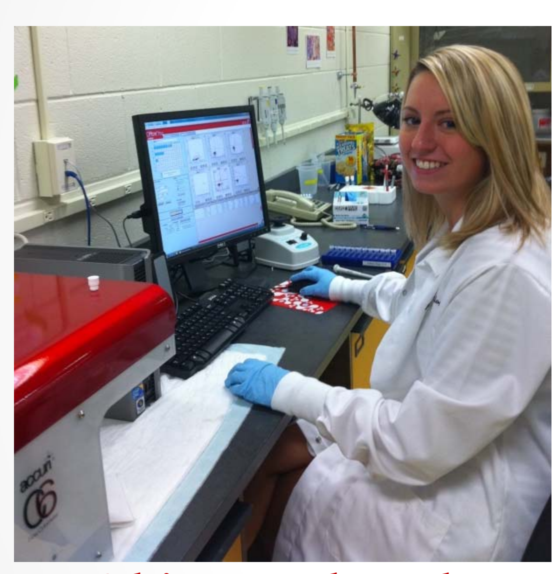
Flow cytometry in undergraduate education



Whitney Edwards

Samantha Scott, Talbot Weston, Sarah Murphy, Melanie Gubbels Bupp – Autumn Immunology Conference 2014

Melanie Gubbels Bupp, Assistant Professor of Biology

General Advice on using cytometry in higher education

- Use the technology for projects that allow students to…
  - more firmly grasp basic concepts
  - test something of interest to them
- Only tell them what they need to know about the technology WHEN they need to know it.
  - Front-loading a lot of technical information is overwhelming and often, ineffective

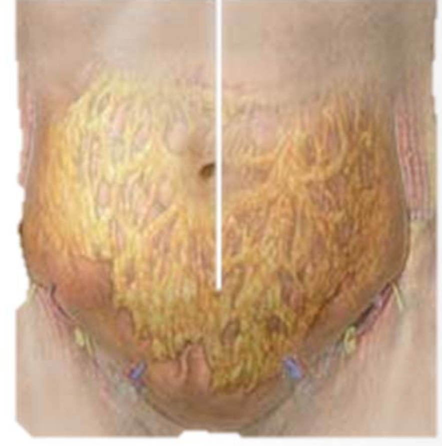
Examples of using cytometry at R-MC

- **Peritonitis Lab** in Immunology course for biology majors [simplified version taught in a freshman mixed (majors/non-majors) lab]
- **Phagocytosis Lab** in Immunology course for biology majors
- **Independent research projects** for undergraduate students

Introduce students to the lab

Thioglycollate-Induced Peritonitis: Recruitment of leukocytes from the circulation, and their subsequent influx into the sites of inflammation is critical for host defense and wound healing. This is a multistep process, which is regulated, in part, by adhesion molecules and chemokines that are upregulated during inflammation. An intra-peritoneal injection of thioglycollate generates local inflammation and initiates the migration of inflammatory cells to the site of inflammation. Thus, thioglycollate-induced peritonitis in mice mimics an acute inflammatory response in the peritoneum.

Peritoneum



Learning Objectives

- Be able to identify particular white blood cells (lymphocytes, monocytes, neutrophils) in a blood smear.
- Explain how and why blood differentials are taken
- Describe the chronological order in which immune cells arrive at sites of inflammation, such as the peritoneal cavity in thioglycollate-treated mice.

Peritonitis Lab Timeline

Learn how to perform and read blood differentials (untreated mice)

Inject mice with TG (1 hr timepoint); Blood differentials on all TG samples; count & stain peritoneal cells from control and TG mice

Finish blood differential analysis; acquire cells on BD Accuri C6; data analysis

Group presentations on lab + discussion questions

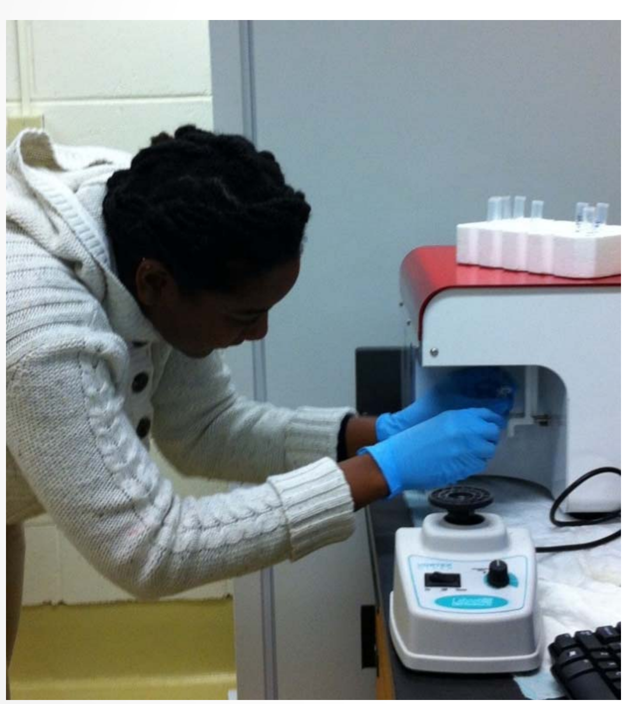
1 2 3 4

Weekly Lab Session

Inject mice with 6% thioglycollate (TG) in PBS or control PBS to induce peritoneal inflammation

R-MC Immunology students acquiring their samples

Victoria Robinson



Lauren Philips, Jane Oh, & Casey Kaufman

Explaining how cytometry works

DN

DP

CD4+

CD8+

Thymus

Labeled antibody specific to CD4

Labeled antibody specific to CD8

Each dot is a cell

CD8+

DP

3.9

81.7

DN

2.8

11.6

CD4+

CD8

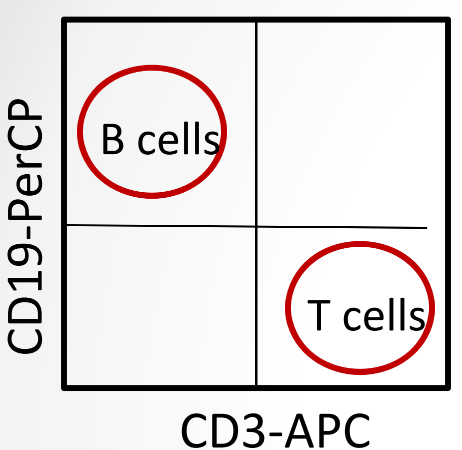
CD4

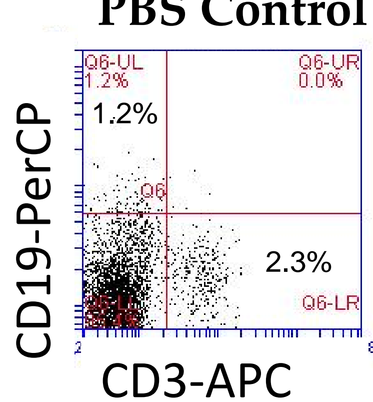
Flow Cytometer

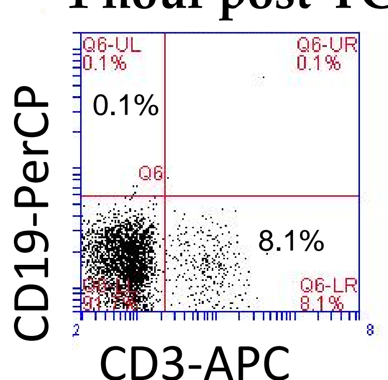
CD8

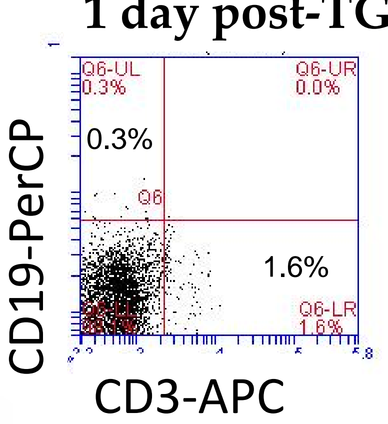
CD4

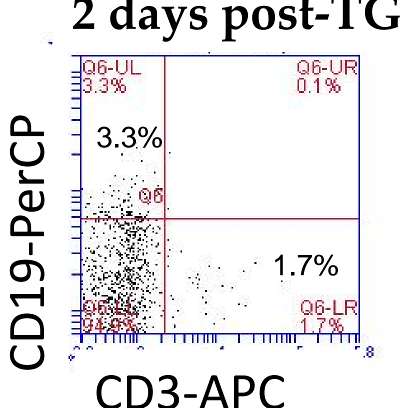
Example student data



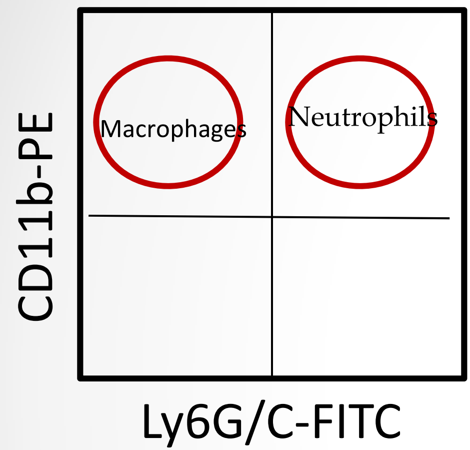


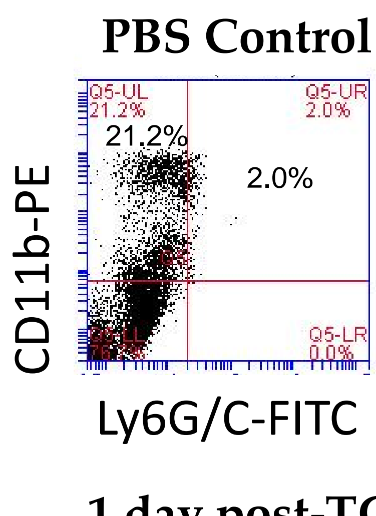


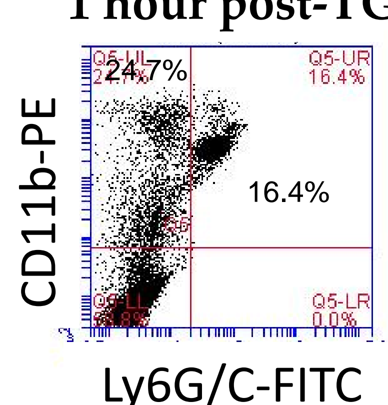


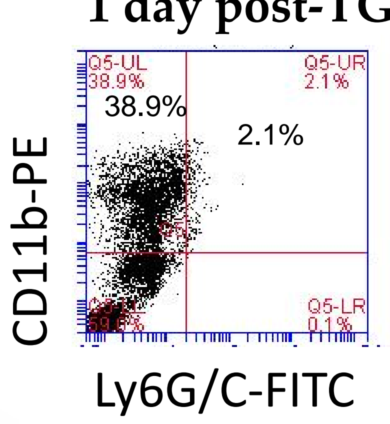


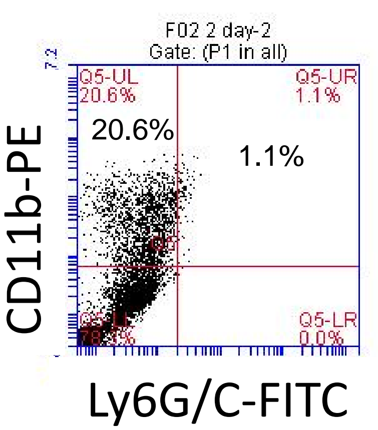
Example student data











Example discussion questions for student peritonitis presentations

- What types of junctions exist between endothelial cells lining blood vessel walls in the non-inflamed, resting condition? How do these junctions change during local inflammation?
- Relate your findings from the blood differentials with your findings from the flow cytometry data. Do the two sets of data "paint the same picture"? Why or why not?
- Map the route newly developed neutrophils must take to enter the inflamed peritoneal cavity. Begin in the bone marrow and end with the peritoneal cavity.

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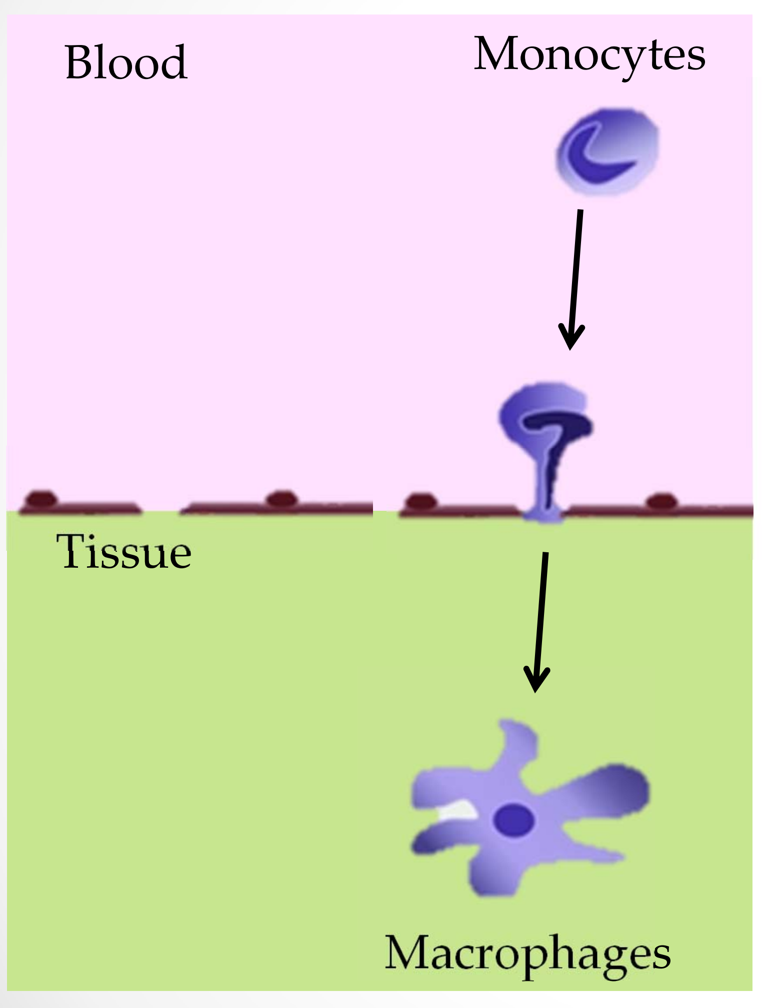
- **Phagocytosis Lab** in Immunology course for biology majors

- **Independent research projects** for undergraduate students

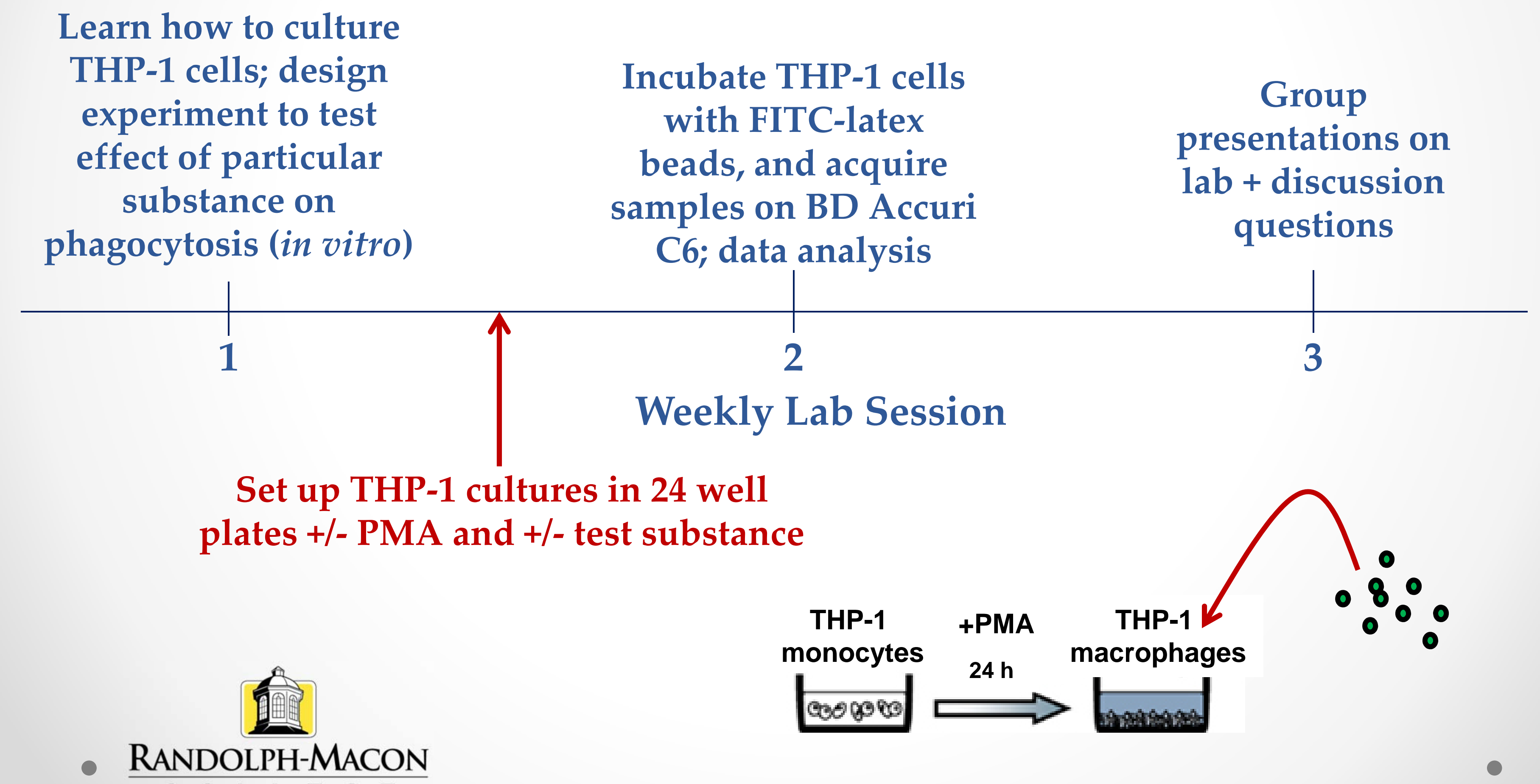
Introduce students to the lab



Learning Objectives

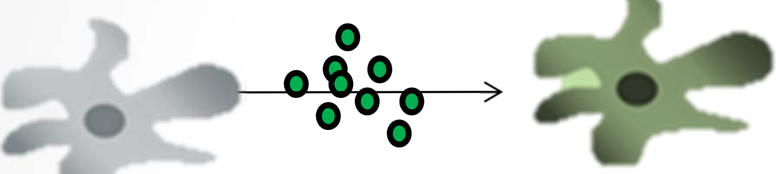
- Compare and contrast monocytes and macrophages
- Evaluate flow cytometry data regarding the ability of cells to phagocytose fluorescently tagged antigens
- Be able to design an experiment to test the impact of various compounds on phagocytosis

Phagocytosis Lab Timeline

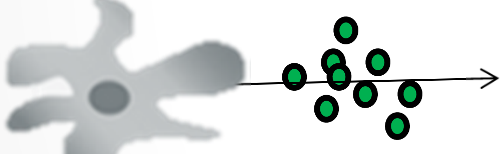


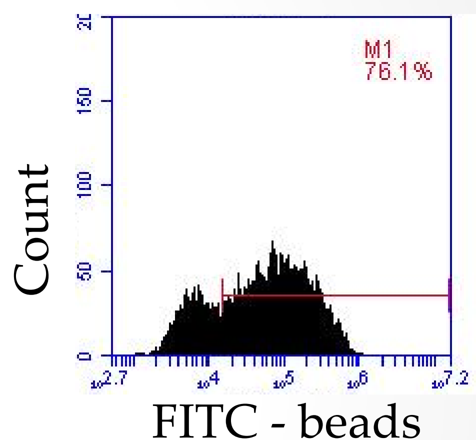
Explaining how cytometry works -- histograms

Lots of phagocytosis

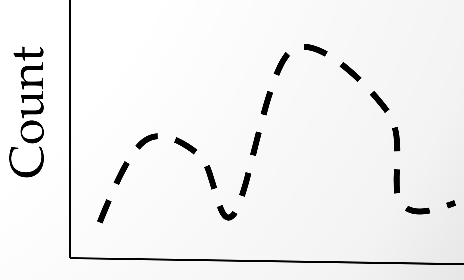


Very little phagocytosis



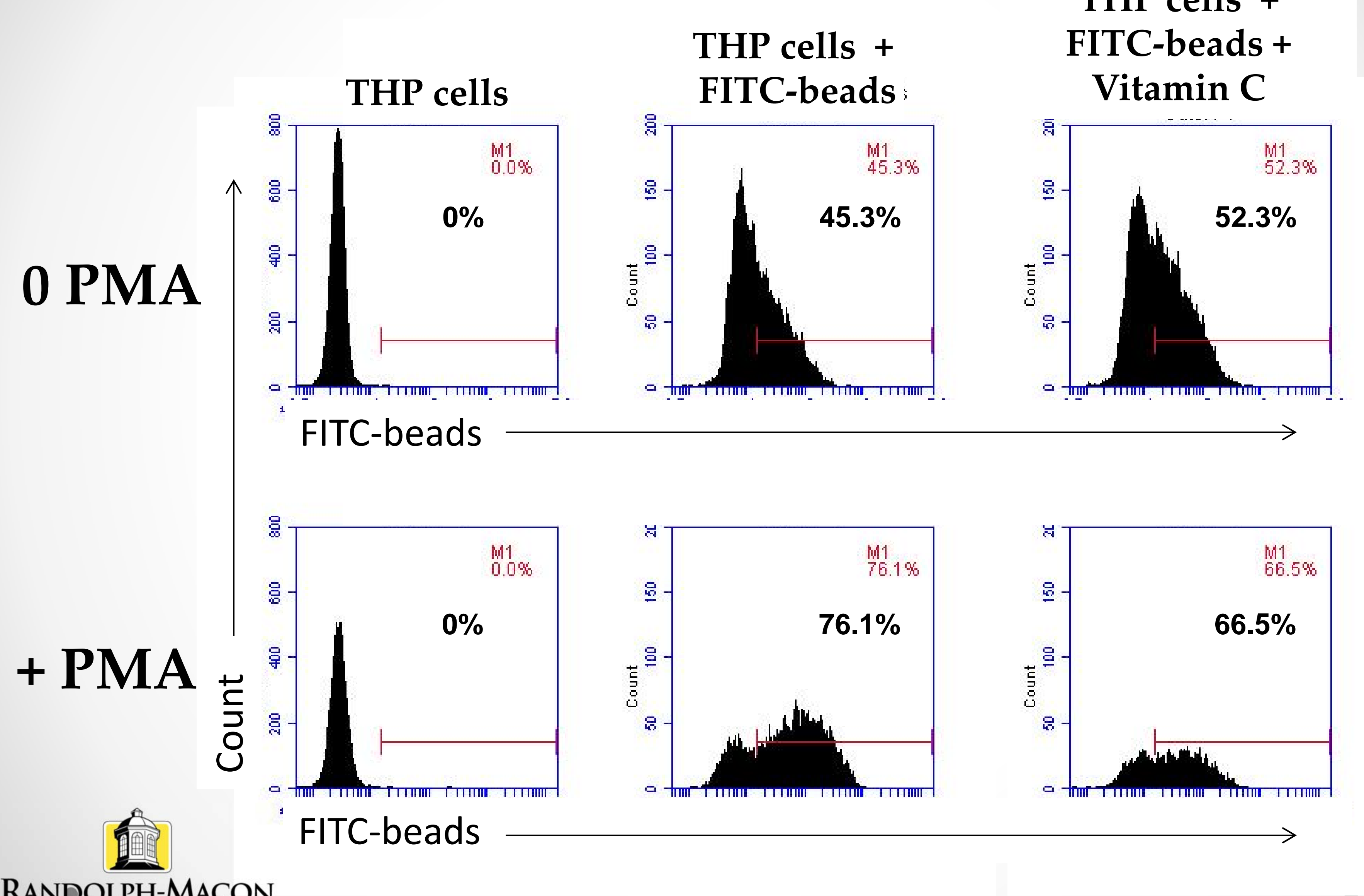


Flow Cytometer



FITC-beads

Example student data



RANDOLPH-MACON COLLEGE

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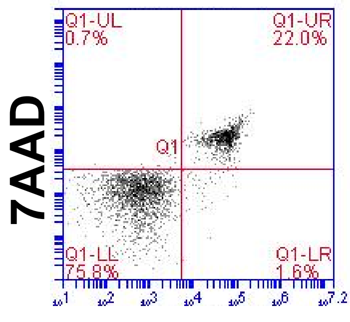
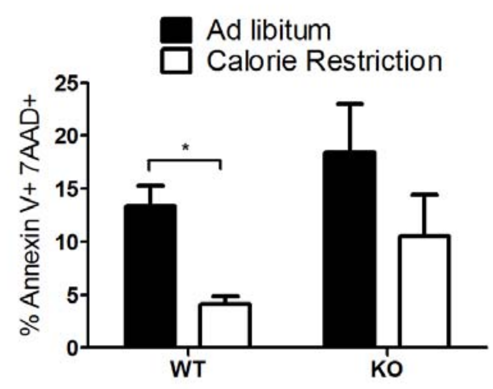
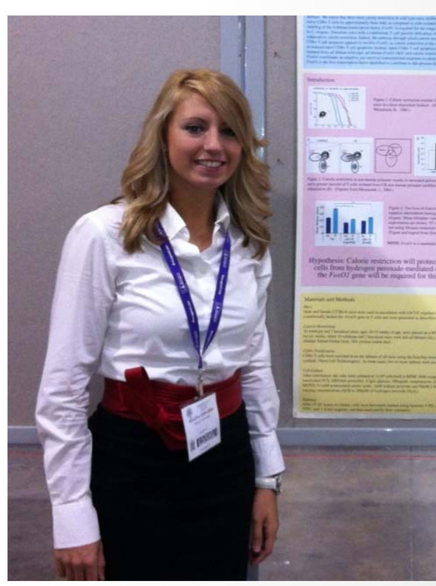
Students at R-MC also use the cytometer in independent research projects

Seth Litvin

Apoptosis Markers



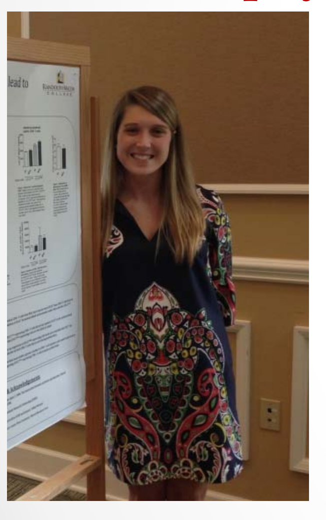




Whitney Edwards

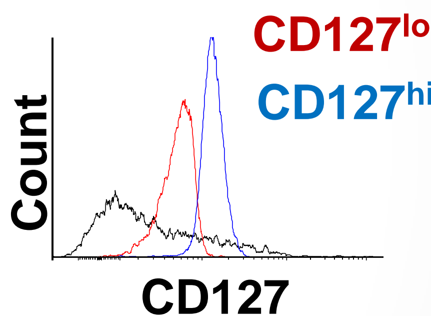
Students at R-MC also use the cytometer in independent research projects

Sarah Murphy

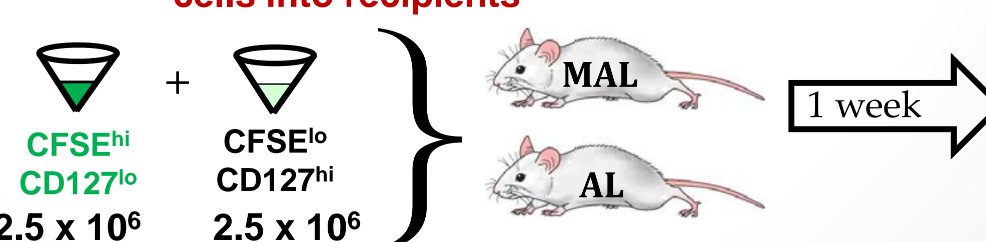


Isolate Naïve CD8+ T cells from Donor mice

Sort them on CD127 expression



Adoptively Transfer cells into recipients



Assess total numbers and CD127 expression of CFSE+ cells by flow cytometry

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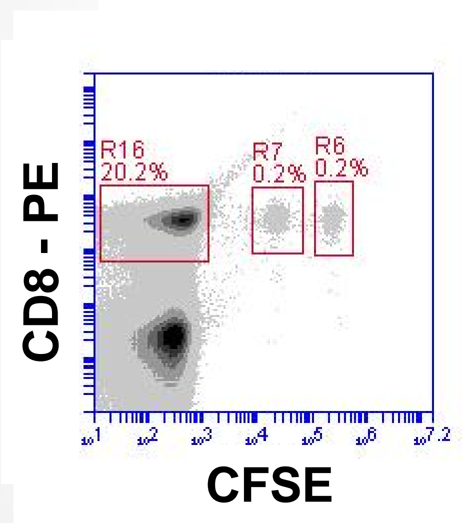
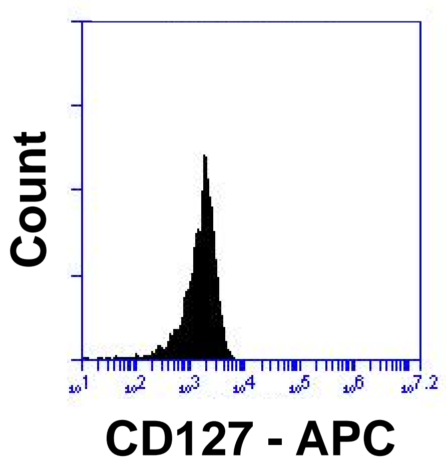
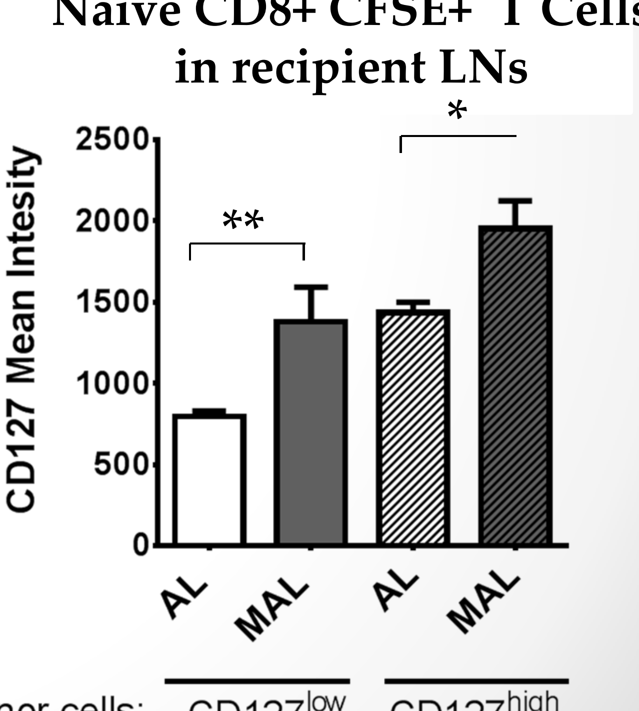






Thank you to R-MC students participating in labs and independent research

- Whitney Edwards
- Erica Horseman
- Rebecca Davis
- Seth Litvin
- Victoria Robinson
- Josh Anoff
- Brittany Mihalcoe
- Alex Koppleman
- Samantha Scott
- Sarah Murphy
- Talbot Weston

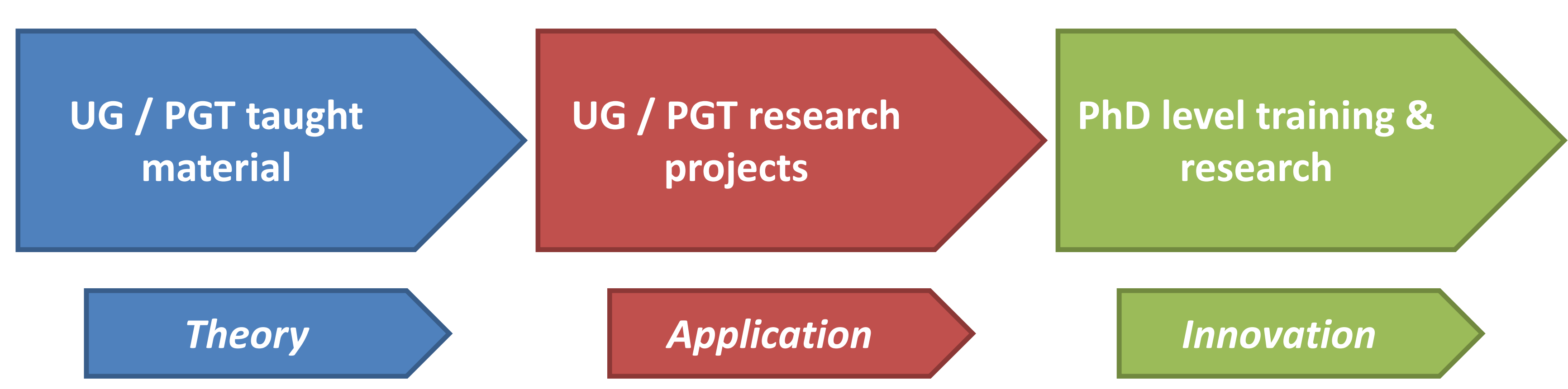
Talbot Weston, Sarah Murphy, and Samantha Scott

Integrating *Microbial* Flow Cytometry Into Education

Tim W Overton
Bioengineering, School of Chemical Engineering
University of Birmingham
t.w.overton@bham.ac.uk *@overtonlab*

How can we get FCM applied in microbiology / microbial biotechnology?

- Collaboration between FCM specialists and microbiology / bioprocessing researchers
- Training at University level

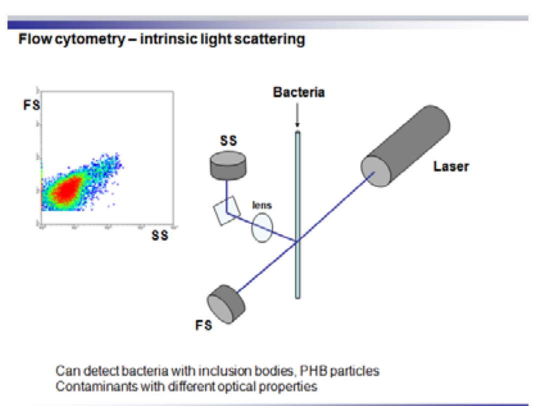


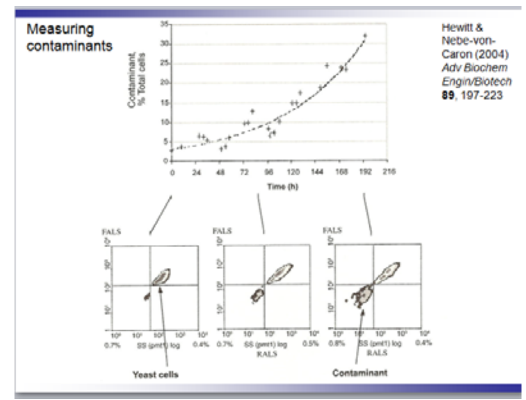
Teaching fermentation

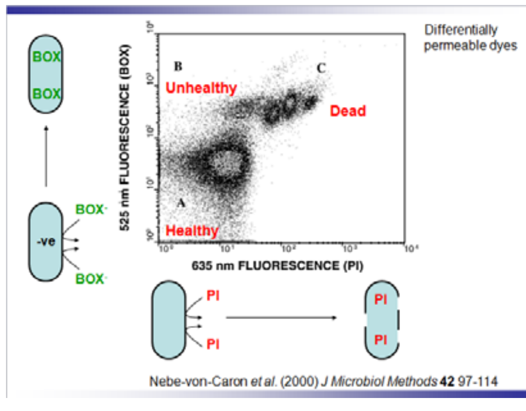
- MSc Biochemical Engineering
  - Fermentation and cell culture for production of biopharmaceuticals
  - Downstream processing of biopharmaceuticals
  - Systems and synthetic biology
  - Pharmaceutical, food and business themes
  - Research project

Teaching analysis of fermentation

- Theory sessions on analytical techniques
  - Online versus offline
  - Real time / non-real time
  - Bulk versus single cell
  - Direct observations
- Theory and advantages of FCM

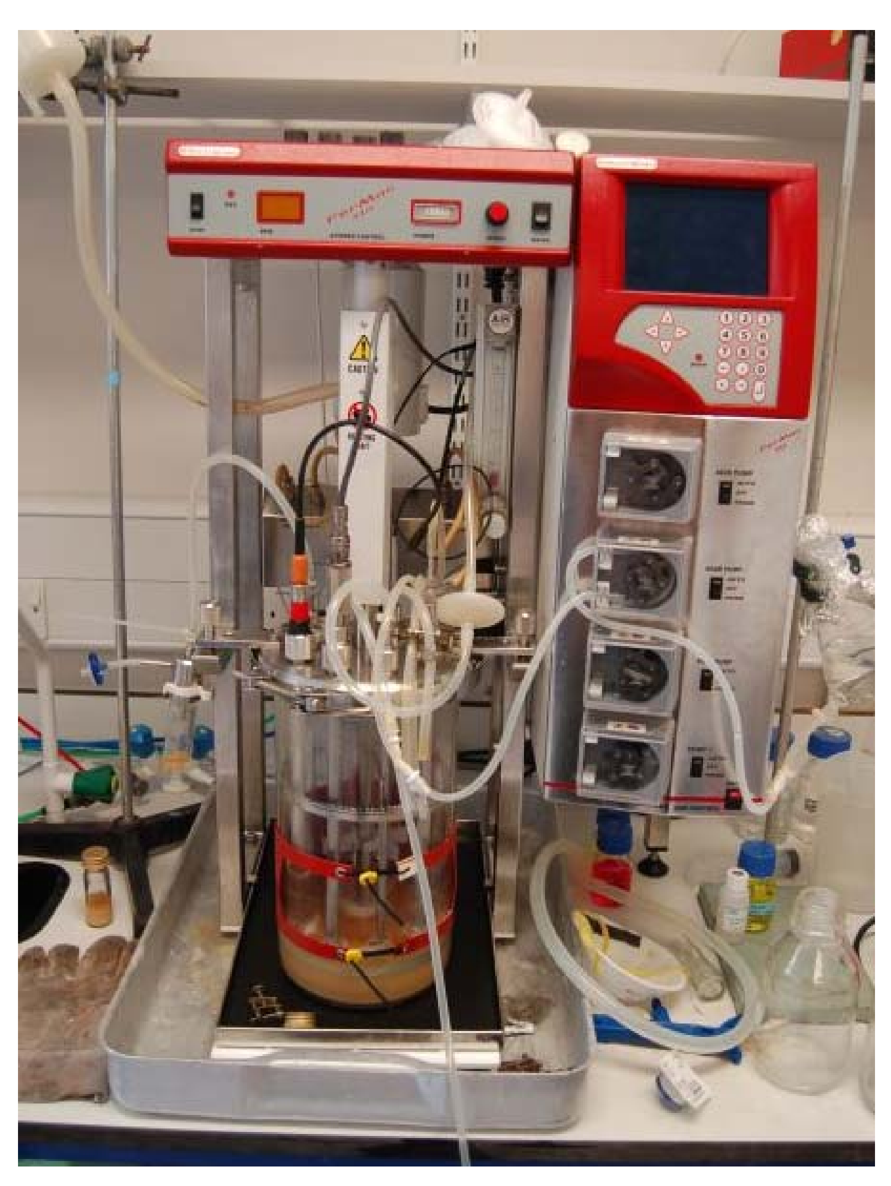




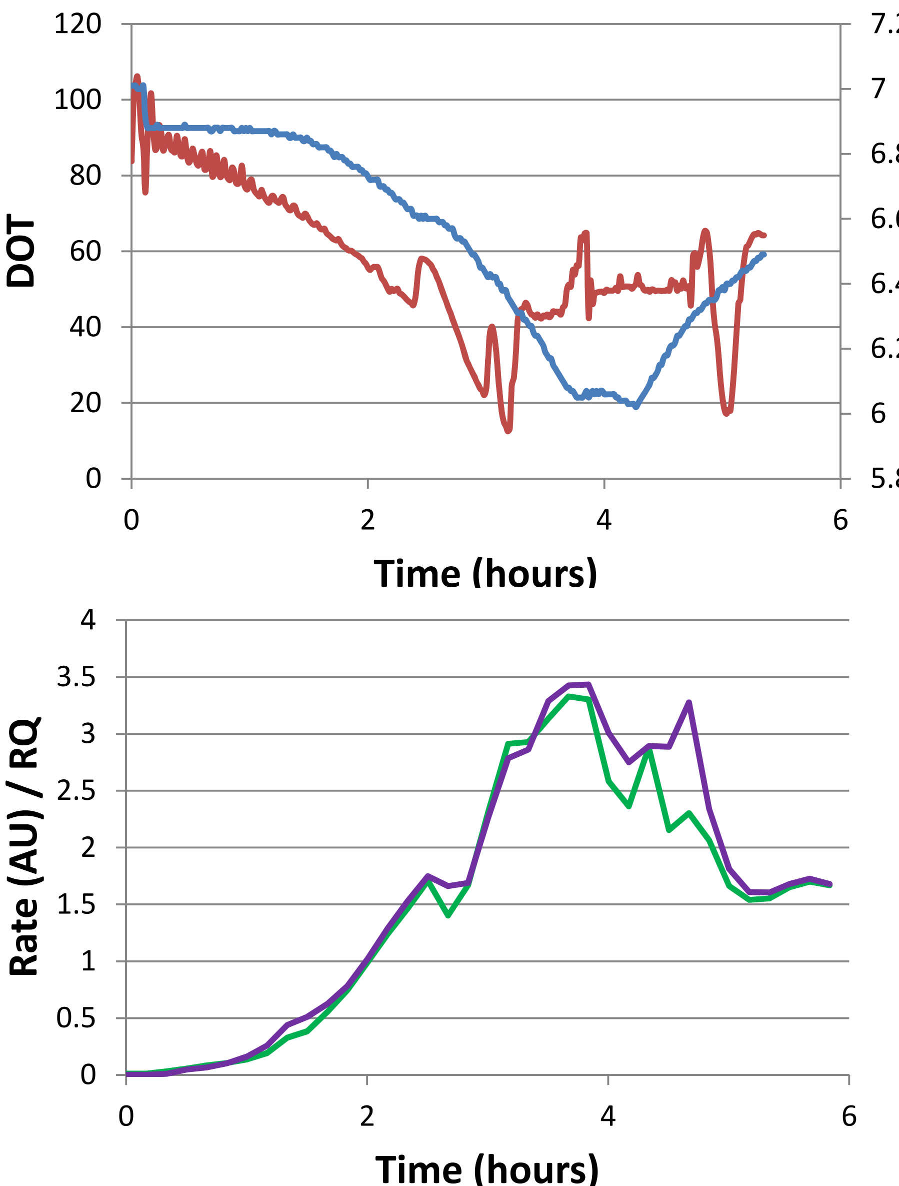


Lab-scale fermentation

- Growth of *E. coli* in 5 litre bioreactors
- Analysis:
  - Traditional techniques: pH, DOT, offgas
  - Biomass measurements:
    - Optical density
    - Colony forming units
    - Dry cell weight
  - FCM



Fermentation data

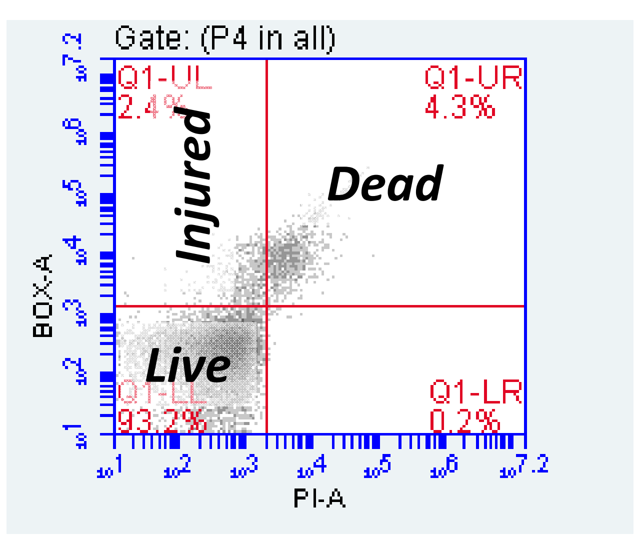


Traditional techniques:
Dissolved O_2 and pH
Basics of growth and physiology

O_2 consumption,
CO_2 evolution
Basics of growth and physiology

FCM with physiology dyes

1 hour



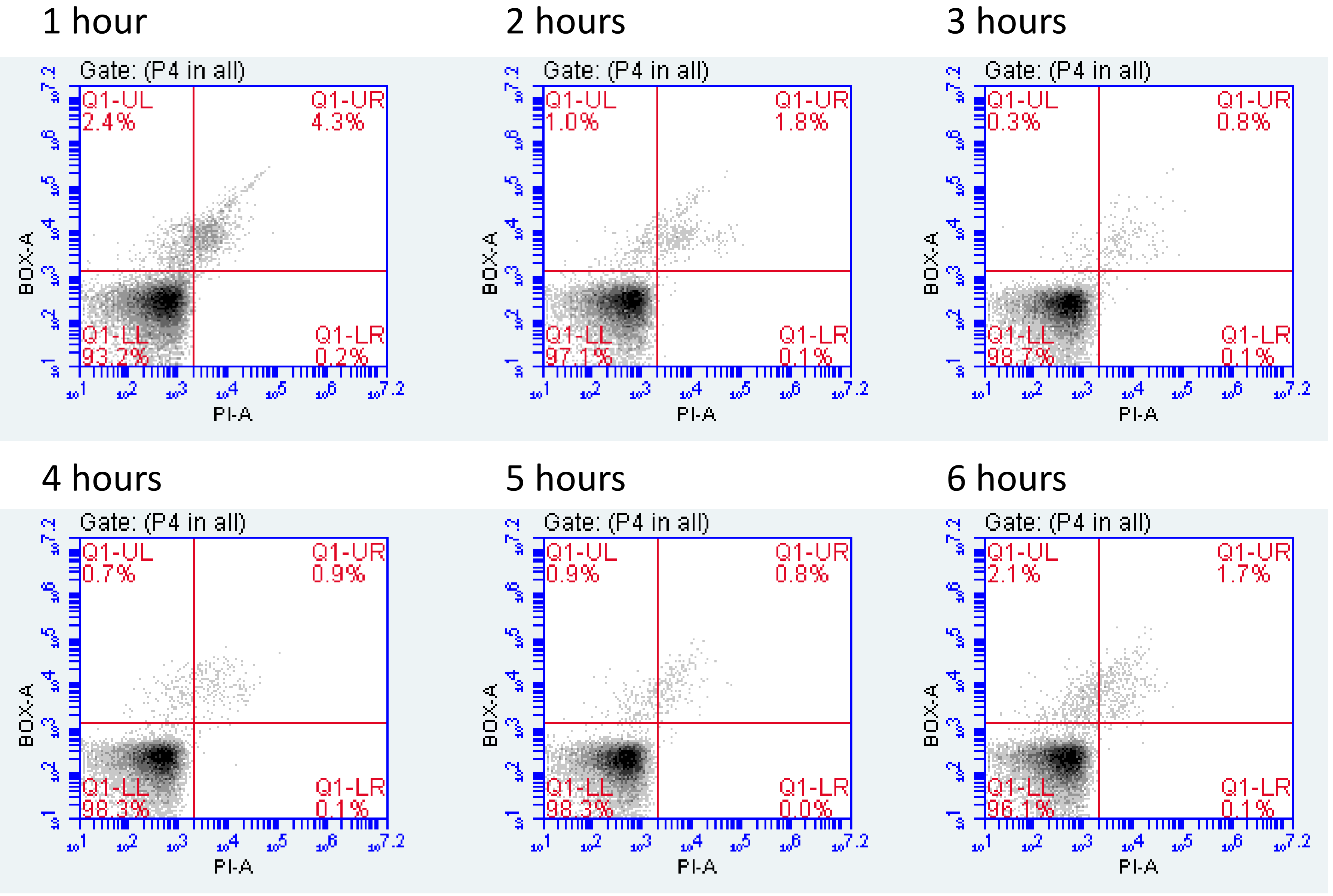
$DiBac_4(3)$ (Bisoxanol; BOX)
Only enters depolarised cells
Stains depolarised cells green

Propidium Iodide (PI)
Enters cells through holes in wall
Stains dead cells red

Advantages:

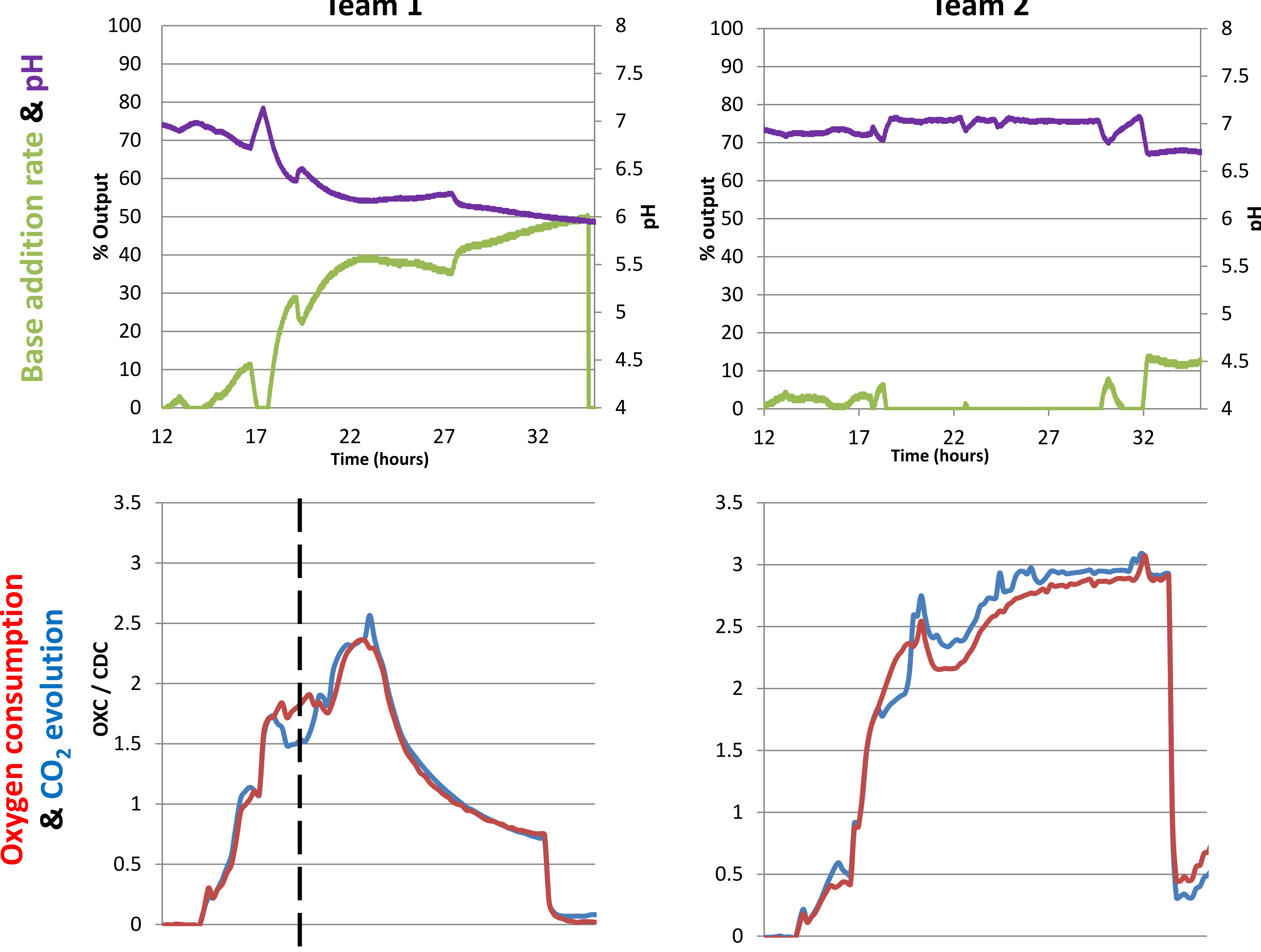
- Rapid method
- Allows monitoring of VBNC bacteria
- Allows counting of cells

FCM with physiology dyes



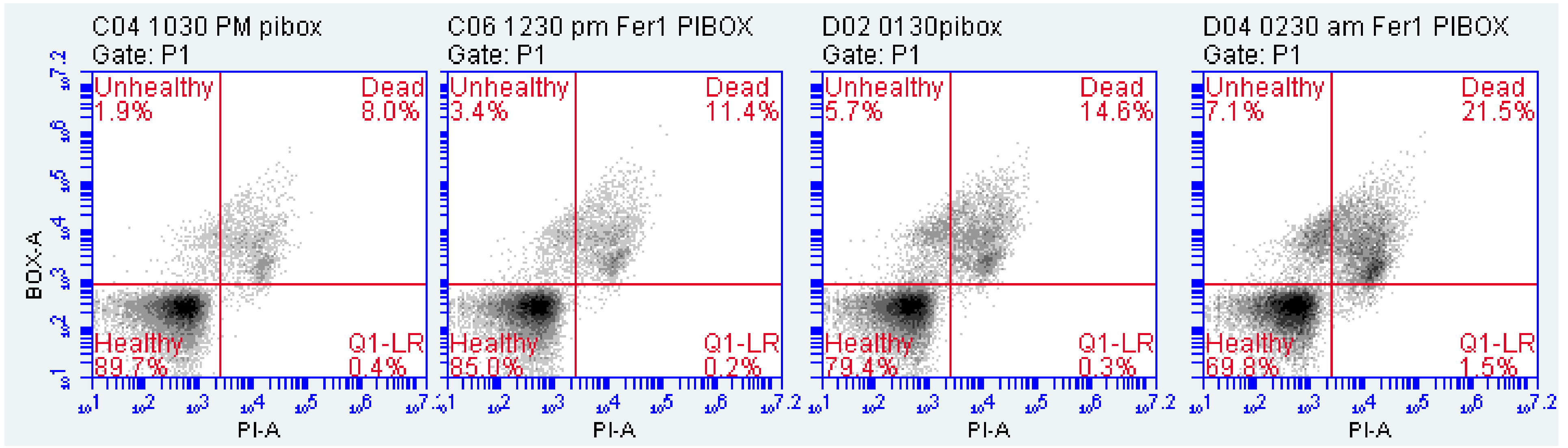
Pilot plant

- Growth of *E. coli* in 150 litre vessels over the course of a week
- Fed-batch with glucose
  - Glucose feeding rate is critical to success
- Students encouraged to develop their own analysis methods and strategies – including FCM


Team 1
Team 2
Base addition rate & pH
% Output
pH
% output
Time (hours)
Oxygen consumption & CO_2 evolution
OXC / CDC
100
90
80
70
60
50
40
30
20
10
0
8
7.5
7
6.5
6
5.5
5
4.5
4
12
17
22
27
32
3.5
3
2.5
2
1.5
1
0.5
0

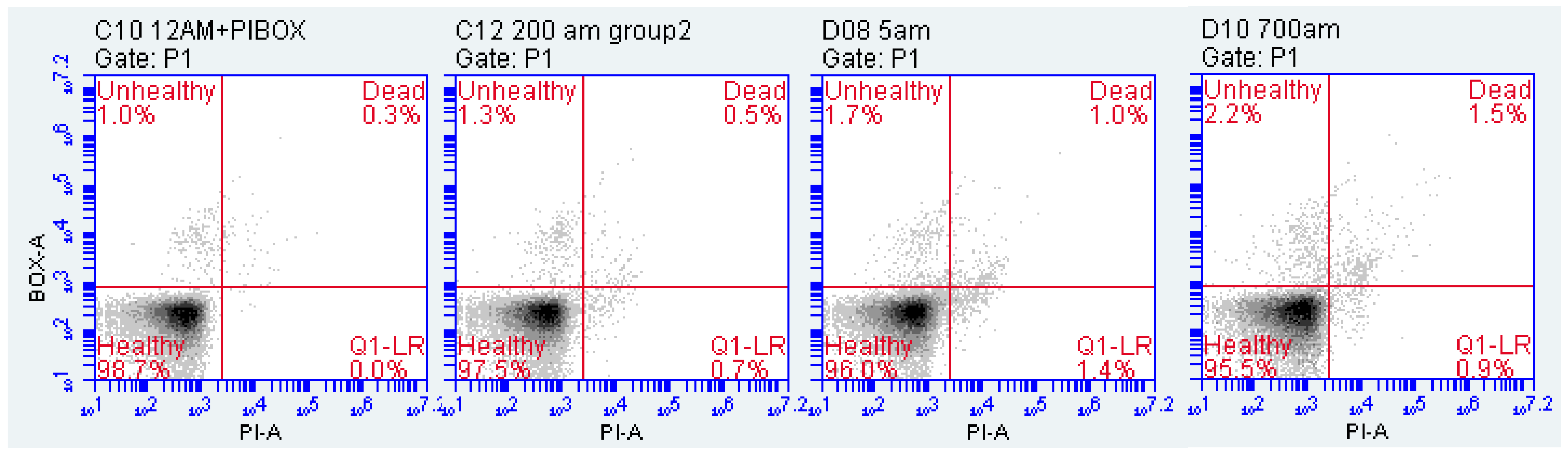
Team 1 – FCM physiology monitoring

More dead and injured cells – reflects poor glucose feed control



Team 2 – FCM physiology monitoring

More healthy cells – good glucose feed control

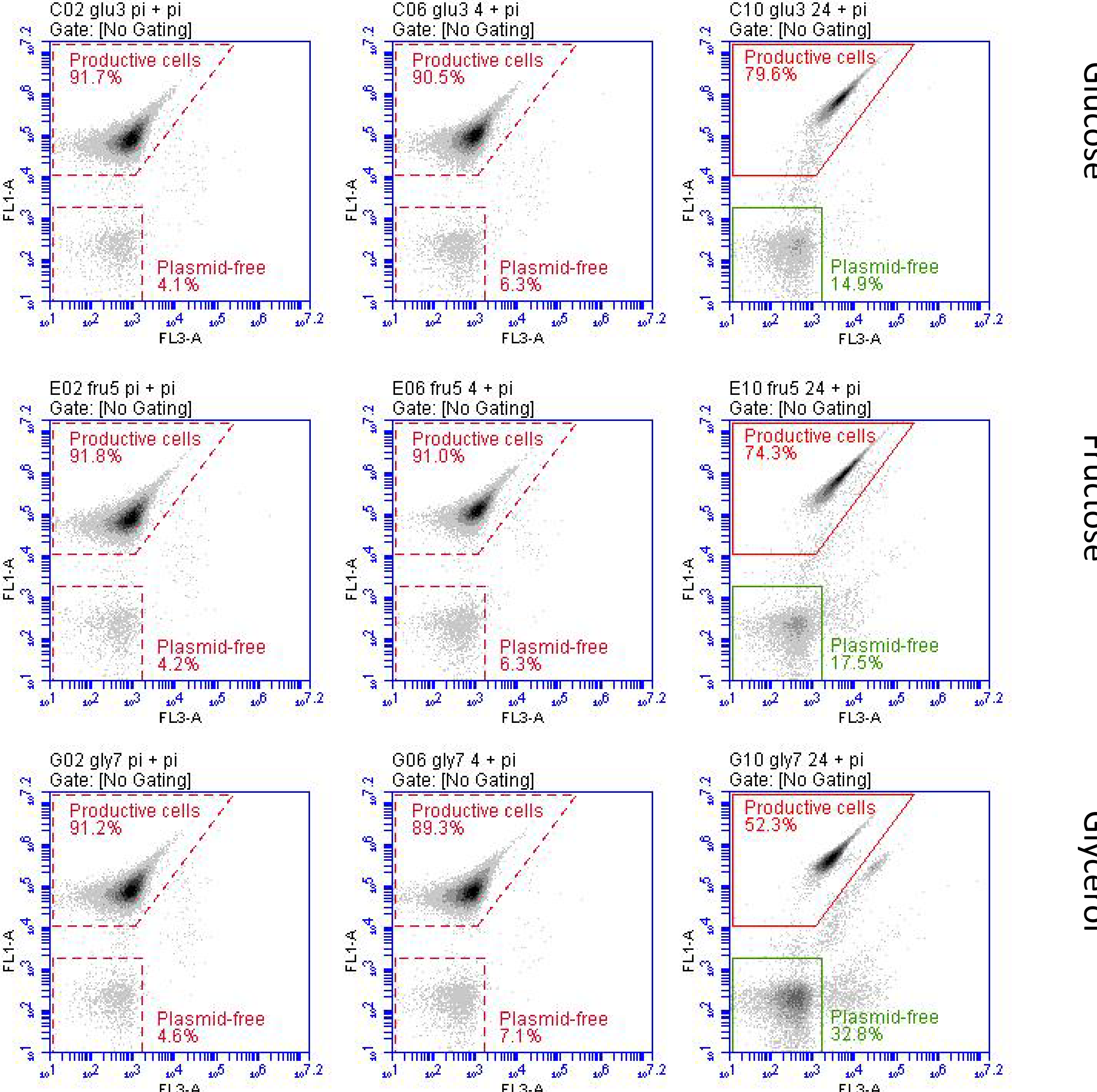


Food microbiology

- Use of flow cytometry as a method for monitoring bacteria during food processing
- Acid resistance and acid adaptation
- Differentiation between bacteria and food matrix

MSc / MEng Research projects

- Monitoring physiology using dyes during biotransformation reactions
- Monitoring poly(3-hydroxybutyrate) (PHB) productivity by bacterial cultures
- Recombinant protein-GFP fusion studies to screen new growth conditions such as choice of carbon source
  - RP-GFP fusions allow measurement of both quantity and folding quality



C02 glu3 pi + pi
Gate: [No Gating]
Productive cells
91.7%
Plasmid-free
4.1%
C06 glu3 4 + pi
Gate: [No Gating]
Productive cells
90.5%
Plasmid-free
6.3%
C10 glu3 24 + pi
Gate: [No Gating]
Productive cells
79.6%
Plasmid-free
14.9%
Glucose
E02 fru5 pi + pi
Gate: [No Gating]
Productive cells
91.8%
Plasmid-free
4.2%
E06 fru5 4 + pi
Gate: [No Gating]
Productive cells
91.0%
Plasmid-free
6.3%
E10 fru5 24 + pi
Gate: [No Gating]
Productive cells
74.3%
Plasmid-free
17.5%
Fructose
G02 gly7 pi + pi
Gate: [No Gating]
Productive cells
91.2%
Plasmid-free
4.6%
G06 gly7 4 + pi
Gate: [No Gating]
Productive cells
89.3%
Plasmid-free
7.1%
G10 gly7 24 + pi
Gate: [No Gating]
Productive cells
52.3%
Plasmid-free
32.8%
Glycerol
FL1-A
FL3-A

Doctoral training

- BBSRC-funded MIBTP (Midlands Integrative Biosciences Training Partnership)
  - Warwick, Birmingham & Leicester Universities
  - 52 PhD students per year
- Extensive training programme:
  - Quantitative skills
  - Placements
  - ***Techniques masterclasses***

Doctoral training - master class

- Day 1:
  - Day of lecture / seminar teaching
  - Ability to go into detail and give examples
  - Papers given to students
- Day 2: Reading / preparation
- Day 3:
  - Students give 10 minute talks on papers
  - Practical session

Doctoral training - practical

- *E. coli* wild-type
- *E. coli* GFP^{+}
- *S. cerevisiae*
- Comparison of scatter measurements
- Live & Dead cells with a variety of stains (PI, BOX, SYTO9)
- Unknown mixtures (problem-solving aspect)
- Hands-on time is invaluable to apply theory (and test theory)

Challenges and opportunities

- Students have diverse backgrounds
  - Gaps in knowledge (e.g. optics, fluorescence)
  - Students can teach each other
- Students are interested in different areas
  - Comparisons with other techniques
  - Fertile ground for new ideas about methods and measurement techniques
- Students are very keen!

Outlook

- Training in theory of FCM is interdisciplinary and needs life science and physical science knowledge.
- Comparison with previously-used techniques is helpful.
- Combination of taught material with practical experience is very useful.

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